Remarks

Claims 49-50, 52-53 and 56-77 are pending. Claims 51 and 54-55 have been newly cancelled. Claims 49-50, 52-53 and 56-57 have been newly amended. Claims 58-77 are newly added. Support for these amendments are found throughout the specification and in the claims as originally filed. No new matter has been entered. All newly added claims are encompassed by Group I of the restriction requirement drawn to methods of identifying biomarkers for rheumatoid arthritis and methods for diagnosis and prognosis of rheumatoid arthritis, further restricted to a CDCA1 gene.

Claims 50 and 67-69 clarify that said levels of RNA encoded by said gene are in blood samples leukocytes which include all of the types of leukocytes in whole blood, i.e. of blood samples which include granulocytes in addition to mononuclear cells (T-lymphocytes, B-lymphocytes and monocytes). This phrase finds clear support in the specification, including at Figure 5C which shows standardized levels of insulin gene expression in each of the fractions of leukocytes which collectively constitute unfractionated leukocytes, i.e. granulocytes, T-lymphocytes, B-lymphocytes and monocytes (labeled "G.R.", "CD 3+", "CD19" and "MONO", i.e., respectively). It is well known to the ordinarily skilled artisan that CD3 and CD19 are specific cell surface markers of T-lymphocytes and B-lymphocytes (refer, for example, to the enclosed Abstract of Casey *et al.*, 1988. simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9). The fact that granulocytes (G.R.), lymphocytes [T-lymphocytes (CD 3+) and B-lymphocytes (CD19+)] and monocytes (MONO) represent all of the types of leukocytes found in blood is taught at Fig. A.23 Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds. (attached) which clearly teaches that leukocytes are composed of granulocytes and mononuclear cells, and that the latter are composed of lymphocytes and monocytes. Additional support for the term "leukocytes" is found at paragraphs [0003] and [0004] and [0087] of the published application (US 20050042630).

New independent claim 70 claims a method of classifying gene expression in a test subject relative to a population of control subjects that includes subjects having rheumatoid arthritis and healthy subjects. New claim 70 comprises a step of quantifying a level of RNA encoded by a

CDCA1 gene in a blood sample from the test subject, and a subsequent step of comparing the level in the sample from the test subject with levels of RNA encoded by the gene in blood samples from the subjects having rheumatoid arthritis and in blood samples from the healthy subjects. The new claim concludes that a statistically significant determination that the level in the sample from the test subject is similar to the levels in the samples from the subjects having rheumatoid arthritis and is different from the levels in the samples from the healthy subjects classifies the level in the sample from the test subject with the levels from the samples from the subjects having rheumatoid arthritis; and that a statistically significant determination that the level in the sample from the test subject is statistically different from the levels in the samples from the subjects having rheumatoid arthritis and is statistically similar to the levels in the samples from the healthy subjects classifies the level in the sample from the test subject with the levels in the samples from the healthy subjects. Support for reciting comparison of biomarker RNA levels of a test subject with those of control subjects having a disease (i.e. rheumatoid arthritis) and with those of healthy control subjects, and determination of a statistically significant difference or similarity therebetween can be found in the published application (US 20050042630), for example at paragraph [0126] ("*When comparing two or more samples for differences, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different levels) were true*"), and at paragraph [0127] ("*When comparing two or more samples for similarities, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different levels) were true*"), respectively. Support for reciting classification of a test subject level relative to spc control levels can be found, for example, at claim 12 as originally filed ("*d) determining whether the level of said one or more gene transcripts of step a) classify with the levels of said transcripts in step b) as compared with the levels of said transcripts in step c)*"), at paragraph [0134] (relating to "*Methods that can be used for class prediction analysis*"), paragraph [0374] ("*Blood samples were taken from patients who were diagnosed with rheumatoid arthritis as defined herein. Gene expression profiles were then analyzed and compared to profiles from patients unaffected by any disease.*").

Claims Rejection - 35 U.S.C. 112 2nd

Claims 51-57 are rejected under 35 U.S.C. 112, 2nd paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

The office action indicates that the recitation of "unfractionated samples of lysed blood" in claim 51 is unclear. Although Applicant respectfully traverses, Applicant has canceled claim 51 and dependent claims 54-55 solely for the purposes of advancing prosecution without prejudice for pursuing the unclaimed subject material in another application, rendering the rejection of claims 51 and 54-55 moot. Applicant has amended dependent claims 52-53 and 56-57 to be dependent from newly added claim 60, which does not recite the phrase "unfractionated samples of lysed blood".

Claims Rejection - 35 U.S.C. 112 1st

Claims 51-57 are rejected under 35 U.S.C. 112, 1st paragraph, as failing to comply with the written description requirement on the grounds that the instantly recited phrase "unfractionated samples of lysed blood" is new matter. Although Applicant respectfully traverses, Applicant has canceled claim 51 and dependent claims 54-55 solely for the purposes of advancing prosecution without prejudice for pursuing the unclaimed subject matter in another application, rendering the rejection of claims 47, 50 and 51 moot. Applicant has amended dependent claims 52-53 and 56-57 to be dependent from claim 49 or newly added claim 60, which do not recite the phrase "unfractionated samples of lysed blood".

Claims 49-57 are rejected under 35 U.S.C. 112, 1st paragraph, as failing to comply with the enablement requirement.

Applicant respectfully traverses. Applicant disagrees with the rejection's assertion that the skilled artisan would have required an undue amount of experimentation to make and/or use the claimed invention in view of the breadth of the claims, the amount of guidance provided by the specification and the level of predictability in the art.

The rejected claims include the steps of determining the level of RNA encoded by an CDCA1 gene in a blood sample obtained from a human test subject and comparing it to the level

of control RNA encoded by the CDCA1 gene in blood samples of control subjects, wherein the comparison is indicative of rheumatoid arthritis in said human test subject.

Applicant specifically traverses the statement on page 4 of the office action that "the independent claim, as written, states that a comparison of a human test subject CDCA1 RNA level in a blood sample to a control indicates that rheumatoid arthritis is present in the test subject", and the statement on page 5 of the office action that the '*The claims are extremely broad because they set forth that any or all comparison between a test subject and RNA level from "control subjects" is indicative of disease*'. Applicant clarifies that the phrase "wherein said comparison of said quantified level of step (a) with said quantified level of said control subjects is indicative of rheumatoid arthritis in said human test subject" of independent claim 49, is a narrowing limitation, limiting the claim to only those comparisons which are indicative of the test subject having rheumatoid arthritis, and excluding those comparisons which do not indicate that the test individual has rheumatoid arthritis.

However, in the interest of expediting prosecution, Applicant has added new claims which more clearly set forth the subject matter of the newly cancelled claims. Specific points raised in the instant enablement rejection will be addressed to the extent that they are relevant to the newly added claims.

The rejection asserts that the claims are broad with respect to "control subjects", indicating that "control subjects" could encompass patients with rheumatoid arthritis, healthy patients, and patients with some other disease such as lupus or a particular stage of rheumatoid arthritis (page 5 of the office action). The instant claims recite two clearly defined sets of controls; patients having rheumatoid arthritis and healthy controls. At least one claim, claim 63, limits the controls to healthy subjects.

The office action states that the claims do not "set forth the direction of the difference necessary to indicate rheumatoid arthritis" (p. 5 of the Office Action) and suggests that without providing this information, the mere observation of differences is an unpredictable indicator of rheumatoid arthritis.

The Applicant respectfully submits that the invention is taught in such terms that one skilled in the art can make and use the claimed invention, including the use of the elected

biomarker CDCA1 as an indicator of rheumatoid arthritis as described in the claims without disclosing the direction or the level of difference that exists between patients having rheumatoid arthritis and individuals not having rheumatoid arthritis. The Applicant has identified the elected gene CDCA1 as differentially expressed as between individuals diagnosed as having rheumatoid arthritis and individuals not having rheumatoid arthritis by demonstrating a statistical difference in the level of RNA, as described in Example 22 and Table 3M. The statistical significance of the differential expression of CDCA1 is evidenced by its P value of .035, as listed in Table 3M. Therefore the Applicant has taught that there is a significant difference in differential expression for CDCA1 as between a population of individuals having rheumatoid arthritis and a population of individuals not having rheumatoid arthritis, and further has taught to compare the level of expression of CDCA1 in a test individual with populations having rheumatoid arthritis and populations not having rheumatoid arthritis using classification methods to determine the similarity or difference in gene expression levels as between the test subject and the tested populations (see paragraphs [0126] to [0127] and [0128] to [0136] in addition to [0374-377]. Independent claims 49 and 70 require that the level of expression of RNA corresponding to CDCA1 be compared with the level of CDCA1 in other individuals who have rheumatoid arthritis and require at a minimum a statistically significant similarity as between the test subject and control subjects having rheumatoid arthritis before the level of gene expression of CDCA1 is considered to be indicative of rheumatoid arthritis or a candidate for rheumatoid arthritis, respectively.

Furthermore, the Applicant contends that it does not require undue experimentation for one of skill to determine the inherent direction or level of the statistically significant differential expression required for the claimed methods of detecting a rheumatoid arthritis, given the widely established and validated analytical tools for analyzing gene expression levels. Therefore, it is not necessary for the Applicant to have taught the exact direction or level of difference between the two populations for one of skill to practice the invention. The Applicant has provided sufficient information by teaching that there CDCA1 is differentially expressed and that the differential expression between healthy and control subjects is significant as between the populations.

The Office Action also suggests that field of analyzing expression profiles "remains highly unpredictable years after the filing of the instant application..." (page 9 of the Office Action) citing Osman et al.. The Applicant submits that the differential expression of CDCA1 as between subjects having rheumatoid arthritis and subjects not having rheumatoid arthritis is, in fact, predictable. In Osman et al., blood cell gene expression profiles of bladder cancer patients (ie.16 individuals having bladder cancer) were compared with 10 healthy individuals. A selection of the genes identified as demonstrating statistically significant difference ($p < 0.05$; page 3376) were tested using RT-PCR on yet an additional sample set of 20 bladder cancer patients and 14 control patients (page 3376, second column) and IGFBP7 continued to verify as a gene which was differentially expressed as between the two populations (see page 3377, second column). As stated in the Manual of Patent Examining Procedure at 2164.03: the "predictability or lack thereof" in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. In the instant application, the disclosed result is a statistically significant differential expression in the level of CDCA1 RNA as between subjects having rheumatoid arthritis and subjects not having rheumatoid arthritis, the statistically significant difference having a p value less than 0.05, as indicated in Table 3M of the instant specification.

The Office Action states on page 7 that Lee teaches that data obtained from gene chips must be replicated in order to screen out false positive results; that Cheung et al. (2003) teaches that there is natural variation in gene expression amongst different individuals (page 9 of the office action); that Wu et al (2001) teaches that gene expression data, such as microarray data, must be interpreted in the context of other biological knowledge, and that the conclusions that can be drawn from a given set of data depend on the particular choice of data analysis, (page 10 of the office action); and that Newton et al. (2001) teaches that a replication of data is required for validation (also page 10 of the office action).

Applicant respectfully disagrees with the contention based on Wu et al. that expression data needs to be interpreted in view of other biological knowledge. Differential gene expression which is reproducible, and is correlated with the state of health or disease of the individual does not necessarily result directly from the state of disease of the individual. Rather these changes in

expression may simply represent a downstream side-effect of pathogenic processes, and it is not necessary that the biological relevance of the data be known to allow this difference in expression to be useful as a biomarker. For example prostate-specific phosphatase and prostate-specific antigen (PSA) were long used as biomarkers without an understanding of their function (refer, for example, to the enclosed abstracts of: Chu TM, 1990, Prostate cancer-associated markers. Immunol. Ser. 53:339-56; and Diamandis EP., 2000, Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin Chem. 46:896-900).

The Examiner also argues, on the basis of post-filing art of Wu (2001) and Newton (2001), that many factors may influence the outcome of the data analysis and notes that conclusions depend on the methods of data analysis. While considerations such as variability, and normalization are of importance, these considerations are well understood by a person skilled in the art and have been applied for many years to permit development of biomarkers which are indicative of disease. These challenges are well understood, as are the routine experiments required to exemplify statistically significant differences in populations.

Applicant notes that the results disclosed by Cheung *et al.* cannot be reliably extrapolated to primary blood samples since the lymphoblastoid cells employed by Cheung *et al.* are significantly modified relative to primary blood cells, due to being cultured cell lines generated by immortalization of primary human cells derived from "CEPH" families, as indicated in Reference no. 10 of Cheung *et al.* (Dausset *et al.*, 1990. Genomics 6:575; enclosed) at p. 575, right column, 1st paragraph. Applicant notes that immortalized cultured cell lines such as the lymphoblastoid cells taught by Cheung *et al.* undergo significant genetic modification such as strong genome-wide demethylation (refer, for example, to enclosed abstract of: Vilain *et al.*, 2003. DNA methylation and chromosome instability in lymphoblastoid cell lines. Cytogenet Cell Genet. 90:93), as a result of extensive *in-vitro* culturing in the absence of immune or apoptotic mechanisms which function to eliminate mutated cells in the body. As such, immortalized CEPH lymphoblastoid cells may represent a particularly unsuitable cell type for modeling gene expression variability in primary blood cells.

To the extent that Cheung et al. could still be considered to suggest that larger populations of diseased and control populations may be useful to determine what level of

differential expression is indicative of disease amongst the population at large, the Applicant submits that the extension of the experiments as outlined in the specification to additional individuals is merely routine. As is noted in Re Wands "*even a considerable amount of experimentation is permissible to practice the claimed methods, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.*" (Re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)).

Furthermore, the decision *In re Angstadt*, 190 U.S.P.Q. 218 (C.C.P.A. 1976) clearly states that even in an unpredictable art, and clearly permits the presence of a screening step to identify those embodiments which possess the desired activity is permissible. In fact, in *Angstadt*, the Court specifically dismissed the notion that the specification must provide a level of guidance that would predict the outcome of an experiment "with reasonable certainty before performing the reaction" and that "such a proposition is contrary to the basic policy of the Patent Act, which is to encourage disclosure of inventions and thereby to promote progress in the useful arts." The "predictability or lack thereof" in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention.

Applicant wishes to point out that in *In re Wands*, the court stated that "[e]nablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. 'The key word is 'undue' not 'experimentation' (citing *In re Angstadt*, 537 F. 2d 498 at 504, 190 U.S.P.Q. 214 at 219 (C.C.P.A. 1976)). The Court also stated that "the test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." (citing In re Jackson, 217 U.S.P.Q. 804 at 807 (Bd. App. 1982)).

As such the Applicants believe there is sufficient guidance provided by the specification that CDCA1 gene is differentially expressed between human individuals who are healthy as compared to those having rheumatoid arthritis, and that the art is sufficiently predictable such

that the amount of experimentation to perform the instantly claimed methods of diagnosing rheumatoid arthritis and identifying candidate subjects who may have rheumatoid arthritis is not undue. In light of the amendments and above remarks, the Applicant contends that the claims are fully enabled, and respectfully requests reconsideration and withdrawal of the instant rejections.

Conclusion

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. No new matter is added. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

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Encl:

Abstract of Casey et al., 1988. simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9;

Abstract of: Chu TM, 1990, Prostate cancer-associated markers. Immunol. Ser. 53:339-56;

Abstract of: Diamandis EP., 2000, Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin Chem. 46:896-900);

Abstract of: Vilain et al., 2000. DNA methylation and chromosome instability in lymphoblastoid cell lines. Cytogenet Cell Genet. 90:93;

Dausset et al., 1990. Genomics 6:575; and

Fig. A.23. Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds.